Lactic Acid Bacteria in the Treatment of Acute Rotavirus Gastroenteritis

Heli Majamaa, Erika Isolauri, *Maija Saxelin, and †Timo Vesikari

*Department of Clinical Medicine, University of Tampere, Finland, *Valio Ltd. R&D, Helsinki, Finland, and †Department of Biomedical Sciences, University of Tampere, Finland*

Summary: We compared different lactic acid bacteria for their effect on the immune response to rotavirus in children with acute rotavirus gastroenteritis. After initial oral rehydration, 49 children aged 6 to 35 months with rotavirus gastroenteritis randomly received either *Lactobacillus casei* subsp. *casei* strain GG (LGG), *L. casei* subsp. *rhamnosus* (Lactophilus), or a combination of *Streptococcus thermophilus* and *L. delbrückii* subsp. *bulgaricus* (Yalacta) twice daily for 5 days. Serum antibodies to rotavirus, total number of immunoglobulin-secreting cells (ISC), and specific antibody-secreting cells (sASC) to rotavirus were measured at the acute stage and at convalescence. The mean (SD) duration of diarrhea was 1.8 (0.8) days in children who received LGG, 2.8 (1.2) days in those receiving Lactophilus, and 2.6 (1.4) days in those receiving Yalacta ($F = 3.3$, $p = 0.04$). The ISC response was comparable in the three study groups, but the rotavirus-specific immune responses were different. LGG therapy was associated with an enhancement of IgA sASC to rotavirus and serum IgA antibody level at convalescent stage. We conclude that certain strains of lactic acid bacteria, particularly LGG, promote serum and intestinal immune responses to rotavirus, and thus may be important in establishing immunity against rotavirus reinfections. Key Words: Diarrhea—Lactobacillus—Immune response.

Lactic acid bacteria, such as is provided in yogurt, are believed to be beneficial in the management of acute infectious diarrhea (1,2). There are a number of species of lactic acid bacteria with different host specificity for colonization. *Lactobacillus casei* subsp. *casei* strain GG (LGG) is of human origin and able to survive passage through the gastrointestinal tract (3). LGG has been shown to promote clinical recovery from rotavirus gastroenteritis in children and enhance intestinal immune responses (4,5). Other commercially available preparations of lactic acid bacteria are also being used for the treatment of acute diarrhea, even though their efficacy has not been formally demonstrated.

There is little information on the immunostimulatory effects of lactic acid bacteria in children. The aim of this study was to investigate the effect of LGG versus commercially available preparations of lactic acid bacteria on local and systemic immune responses, by measuring the antibody-secreting cells in peripheral blood with the solid-phase enzyme-linked immunospot assay (ELISPOT) and analyzing the serum rotavirus IgA antibodies using an enzyme-linked immunosorbent assay (ELISA) method.

Address correspondence and reprint requests to Dr. Erika Isolauri, Department of Clinical Medicine, University of Tampere, P.O. Box 607, 33101 Tampere, Finland.

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MATERIALS AND METHODS

Patients and Study Design

The study was carried out between February and June 1993, during a rotavirus epidemic. Within the study period, 60 children between 4 and 35 months of age were admitted for acute gastroenteritis of <7 days' duration at the Department of Pediatrics, Tampere University Hospital, Finland, and enrolled in the study. Rotavirus-negative patients were subsequently excluded; rotavirus-positive patients who had had three or more watery stools in

the last 24 h before admission were included in the study. The final study population consisted of 49 patients (25 boys and 24 girls) with a mean age of 18 (SD 9) months. Apart from the randomized study, five patients not receiving lactobacilli were studied separately for immune responses to rotavirus using the same methods as in the main study.

On admission, the children were weighed and examined clinically. The degree of dehydration (%) was determined from acute weight loss and clinical signs. The patients were orally rehydrated within 6 h (6,7). If diarrhea continued, the losses were replaced with the rehydration solution and free water. Upon admission, a blood sample was taken for the analysis of serum sodium and potassium levels and blood acid-base balance.

After rehydration, alimentation appropriate for age was resumed, excluding only fermented milk products. Infants who were still totally breast-fed were not included in the study. The children were randomly and double-blindly allocated to receive one of the three lactic acid bacteria preparations twice daily for 5 days. The first group (n = 16) was given freeze-dried LGG; the second group (n = 14) was given *Lactobacillus casei* subsp. *rhamnosus* (Lactophilus); and the third group (n = 19) was given a combination of *Streptococcus thermophilus, Lactobacillus delbrückii* subsp. *bulgaricus* and *L. casei* subsp. *rhamnosus* (Yalacta). The preparations were stored at −18°C in 1.25-g doses; each dose was kept in a small plastic bag. Ten doses for each patient were kept in an envelope. A running number for each patient was marked on the envelope and on each bag. The preparations all looked similar. Before administratation, the dry powder was first mixed with 5 ml of water and then given with food or via nasogastric tube.

For confirmation, the *Lactobacillus* strains were isolated on MRS agar (Lab m, U.K.) and from Yalacta powder on $MRS_{pH5.4}$ and M17 agar (IDF standard 117A: 1988). The taxonomy of the strains was confirmed by API 50 CHL (Bio Mérieux, France) according to the instructions of the manufacturer. APILAB Plus (v.3.1.1., 1990) software was used to give the final identification. LGG is a human strain used in dairy products in Finland (8). Based on carbohydrate fermentations, it was shown to belong to *L. casei* subsp. *casei*. The freeze-dried powder of LGG was diluted with microcrystalline cellulose (Ph. Eur. 2nd ed.II) to a concentration of 5×10^9 cfu/g (supplied by Valio Ltd., R&D, Helsinki, Finland). Lactophilus is a commercial pharmaceutical powder produced by Laboratoires Lyocentre, France, and supplied in Finland by Oy Organon Ab, Finnpharma. The powder was declared to contain *L. acidophilus* strain 1×10^9 cfu/g, but it actually contained *L. casei* subsp. *rhamnosus*. Two separate production lots containing 2.5×10^8 and 4.4×10^7 cfu/g, respectively, were used and mixed together to contain 2.2×10^8 cfu/g. Yalacta (bande bleu), a traditional yogurt starter culture, was purchased from Yalacta, Caen Cedex, France. It contained 2.8×10^9 cfu/g of lactic acid bacteria; 95% of the culture was *Streptococcus thermophilus;* 4% was *L. delbrückii* subsp. *bulgaricus;* and 1% was *L. casei* subsp. *rhamnosus*, respectively.

The attending nurses assessed the quality (characterized as watery, loose, or solid) and number of stools and vomiting episodes. The patients were discharged according to the clinical judgment of the attending physician. A medical examination was done 3 weeks later; in the meantime, the parents were asked to contact the investigators if diarrhea recurred.

Blood specimens were taken for the ELISPOT assay and for the determination of serum rotavirus antibodies on the day after admission and 3 weeks later. A stool specimen was collected on the 1st day in the hospital and tested for rotavirus antigen with an enzyme immunoassay (Rotazyme, Abbott Laboratories, North Chicago, IL, U.S.A.).

Immunological Studies

ELISPOT is a solid-phase ELISA technique for the enumeration of immunoglobulin-secreting cells and specific antibody–secreting cells (9–11). The number of these lymphocytes in blood reaches a maximum 1 week after oral administration of antigen (12,13). The total number of ISC and sASC were determined as described previously (13).

In brief, for determination of sASC, flat-bottomed microtiter plates were first coated with rabbit anti-rotavirus immunoglobulin (major activity directed to VP6-antigen of group A rotaviruses) and the next day with rotavirus antigen (Behringwerke AB, Marburg, Germany). Uncoated binding sites were blocked with 1% bovine serum albumin in PBS. Next, the lymphocyte suspension was applied and incubated for 2 h at 37°C. Thereafter, alkaline phosphatase–conjugated anti-human IgA, IgG, or IgM-serum was added to detect the secreted antibodies. The antibodies were visualized by application of enzyme-labeled antisera followed by a hot

substrate agarose overlay and by counting of colored spots. To determine the number of ISC, rabbit anti-human IgA, IgG, and IgM (Dakopatts a/s, Glostrup, Denmark) were used as coating antigens. Subsequent steps were the same as in the determination of the sASC.

Rotavirus IgA serum antibodies were measured using an ELISA method, which was a single serum dilution modification of the test described by Midthun et al. (14). The antigen was a rhesus-human reassortant rotavirus D x RRV grown in MA-104 cells; this virus has the VP7 serotype specificity of human rotavirus serotype 1. Briefly, microtiter plates were coated with hyperimmune rabbit antirotavirus serum (Dakopatts a/s, Roskilde, Denmark) and subsequently with the supernatant of D x RRV-infected MA-104 cell culture. Known positive and negative control sera were included. The results were expressed in enzyme immune units (EIU) after comparison to positive and negative control sera. The EIU value of a given serum was its percentage of the absorbance value of the positive reference serum corrected by background.

Statistical Analyses

Analysis of variance (ANOVA) was used for intergroup differences. Differences in proportions were evaluated with the chi-square test. Because of skewed distribution, natural logarithmic transformations were used (15) for the numbers of ISC and sASC as well as rotavirus serum antibodies, and data are reported as geometric means with 95% confidence intervals (CI). The successive ISC and sASC measurements were compared with the paired *t* test.

Ethical Considerations

The study protocol was approved by the Ethical Review Committee of Tampere University Hospital. Informed consent was obtained from the parents of the enrolled children.

RESULTS

The three patient groups did not differ significantly in clinical features of diarrhea at the time of admission (Table 1). The mean duration of diarrheal symptoms was ~2 days, and on average there was mild to moderate isosmolal dehydration and metabolic acidosis. The ages of the patients in the study groups were comparable.

Clinical Outcome

All patients were rehydrated with an oral rehydration solution. Six patients needed additional intravenous fluid therapy. Rapid refeeding was carried out without difficulty in all cases.

The duration of diarrhea was significantly different between the study groups; those receiving LGG had a shorter duration of diarrhea than those receiving other preparations (Table 2). The mean (SD) duration of diarrhea was 1.8 (0.8) days in children who received LGG, 2.8 (1.2) days in those receiving Lactophilus, and 2.6 (1.4) days in those receiving Yalacta ($F = 3.3$, $p = 0.04$). The mean (SD) duration of diarrhea in an untreated comparison group ($n = 5$) was 2.6 (1.3) days. The effect of LGG on the duration of diarrhea was not manifest on the 1st day; however, after 2 days of treatment, only 19% of patients receiving LGG had diarrheal stools, whereas 64% of patients receiving Lactophilus and 58% of patients receiving Yalacta continued to have watery diarrhea (Table 2). All patients recovered from diarrhea within 6 days, and no patient required rehospitalization.

Immunological Studies

The total numbers of IgA, IgG, and IgM ISC were enhanced at the acute stage of diarrhea, as com-

TABLE 1. *The clinical characteristics of patients on admission*

	LGG (n = 16)	Lactophilus (n = 14)	Yalacta (n = 19)	ANOVA p
Age (mo)	21.3 (9.5)	19.4 (8.4)	16.6 (9.2)	0.31
Duration of symptoms at home	2.2 (1.7)	2.3 (2.3)	2.5 (1.7)	0.89
Dehydration (%)	4.8 (2.2)	3.8 (0.8)	4.4 (1.5)	0.38
Temperature (°C)	38.5 (0.8)	38.6 (1.3)	39.0 (1.4)	0.37
S-Na^+ (mmol/L)	137 (3)	139 (3)	138 (4)	0.55
S-K^+ (mmol/L)	4.0 (0.4)	4.1 (0.5)	3.9 (0.3)	0.37
Blood pH	7.39 (0.05)	7.37 (0.06)	7.37 (0.03)	0.23
Base excess (mmol/L)	-5.8 (2.6)	-7.7 (3.4)	-6.4 (3.0)	0.27

Data denote means (SD).

TABLE 2. *Mean (SD) duration of diarrhea in hospital, number (%) of patients having watery diarrhea, and number of patients vomiting 1, 2, and 3 days after treatment*

	LGG (n = 16)	Lactophilus (n = 14)	Yalacta (n = 19)	p
Duration of diarrhea (days)	1.8 (0.8)	2.8 (1.2)	2.6 (1.4)	0.04[a]
Watery diarrhea				
Day 1	11 (69)	13 (93)	13 (68)	0.20[b]
Day 2	3 (19)	9 (64)	11 (58)	0.02[b]
Day 3	0 (0)	3 (21)	6 (32)	0.05[b]
Vomiting				
Day 1	10	5	9	0.34[b]
Day 2	0	4	2	0.05[b]
Day 3	0	1	2	0.42[b]

For comparison, mean (SD) duration of diarrhea in untreated children (n = 5) was 2.6 (1.3) days.
[a] ANOVA.
[b] Chi-square test.

pared to the convalescent stage (p = 0.03, p = 0.0003, p = 0.05, respectively). This pattern of ISC response and the numbers of ISC were comparable in the study groups. At the acute stage of diarrhea, IgA ISC/10^6 cells were 873 (95% CI 512–1489) in the LGG group, 540 (267–1091) in the Lactophilus group, and 1094 (469–2553) in the Yalacta group (p = 0.31). At convalescence, the respective numbers were LGG: 313 (178–548), Lactophilus: 482 (206–1128), Yalacta: 605 (357–1023) (p = 0.23).

During the acute stage of rotavirus gastroenteritis, rotavirus-specific IgM sASC/10^6 cells [LGG: 6.5 (0.8–49.7), Lactophilus: 4.5 (0.3–66), Yalacta: 6.2 (0.8–45.4)] were not different between the groups (p = 0.96), nor did they differ at convalescence [LGG: 0.7 (0.1–6.7), Lactophilus: 0.4 (0–10.9), Yalacta: 1.8 (0.2–19.5)] (p = 0.68). Likewise, IgG sASC were comparable in the three groups at the acute stage, as follows: LGG: 4.7 (0.3–72.8), Lactophilus: 25.8 (2.2–296), Yalacta: 35.1 (13.6–90.2) (p = 0.24). At convalescence, the IgG sASC value in the LGG group was slightly, but not significantly, higher than in the other two groups, as follows: LGG: 10.8 (4.2–27.7), Lactophilus: 0.5 (0–13.8), Yalacta: 0.8 (0–38.9) (p = 0.08).

The number of IgA sASC to rotavirus at convalescence was higher in patients receiving LGG than in those receiving Lactophilus or Yalacta (Table 3). Most patients (10 of 11) receiving LGG showed a detectable rotavirus IgA sASC response at the convalescent stage, whereas three of seven patients receiving Lactophilus and two of seven patients receiving Yalacta had a detectable sASC response.

All patients had a detectable response in serum rotavirus IgA ELISA antibodies. The mean serum rotavirus IgA antibody levels at convalescent stage were higher in the LGG and Lactophilus groups than in the Yalacta group (Table 3).

TABLE 3. *The number of IgA sASC/10^6 cells to rotavirus and mean levels (95% CI) of serum IgA antibodies (EIU) to rotavirus during the acute and convalescent stage of rotavirus gastroenteritis*

	LGG	Lactophilus	Yalacta	ANOVA p
sASC response				
acute	0.1 (0–2.3)	0.2 (0–3.1)	0.5 (0–7.2)	0.70
convalescent	3.3 (0.9–12.7)	0.1 (0–3.2)	0.1 (0–1.5)	0.01
serum IgA antibodies				
	(n = 13)	(n = 11)	(n = 12)	
acute	0.3 (0.1–1.7)	0.3 (0–1.6)	0.1 (0–0.3)	0.41
convalescent	27.5 (18.9–40)	29.6 (21.1–41.4)	6.1 (1.4–26.1)	0.01

For comparison, sASC response (>1 sASC/10^6 cells) was detected in one of five untreated controls at the acute stage and in none of five at convalescence.

DISCUSSION

The results of our study confirm previous findings that LGG promotes clinical recovery from acute gastroenteritis and potentiates gut immune response to rotavirus (4,5). They also suggest that LGG differs from other strains of lactic acid bacteria in this respect.

Preparations of lactic acid bacteria are commonly used for the management of acute diarrhea, even though there is little proof of their efficacy and limited information on the mechanisms of action. There are a number of species of lactic acid bacteria with different host specificity for colonization. Randomized comparative studies on the efficacy of different strains of lactic acid bacteria are scanty and heterogenous regarding the etiology of diarrhea, strains of bacteria, and dosage and dosage schedules (1,2,16–20).

In this study, we administered lactic acid bacteria orally to patients with rotavirus gastroenteritis to compare the effects of LGG with commercially available preparations. We found that the duration of diarrhea was shorter in patients receiving LGG than it was in untreated controls and patients receiving Lactophilus or Yalacta. LGG therapy was also associated with an enhancement of rotavirus-specific IgA-secreting cells and serum IgA rotavirus antibodies at convalescent stage. Lactophilus-treated children showed an increase of serum rotavirus IgA antibodies, as compared with the group receiving Yalacta. Perdigón et al. have reported enhancement of the synthesis of IgA in mice receiving *Lactobacillus casei* (21,22).

Even though LGG enhances specific immune responses to rotavirus, the mechanisms behind this phenomenon and its relation to the clinical benefit remain unclear. Among the possible mechanisms responsible for the favorable clinical response are the stabilization of the mucosal barrier and reinforcement of the disturbed intestinal microecology (23–25). One explanation might be that the ability of lactic acid bacteria to survive in the gastrointestinal tract varies (26,27). LGG is known to adhere to the intestinal epithelium and give rise to transient colonization of the gut (3).

Generally, the use of lactic acid bacteria is based on the premise that these preparations can reinforce the gut microflora, an important component of the defense barrier (24,25,28,29). In acute gastroenteritis, the barrier function and intestinal microecology are disturbed (30–34). Lactic acid bacteria may prevent the growth of enteric pathogens by producing antimicrobial substances, by competing for either adhesion receptors or nutrients, or by stimulating immunity (24). In a study of rotavirus enteritis in infant mice, Heyman et al. (29) found that enhanced intestinal permeability was more marked in the absence of intestinal microflora than in its presence. We have recently shown, in a suckling rat model, a decline in intestinal permeability after dietary introduction of LGG (23,35). Simultaneously, the immune response increased significantly. This finding suggests a link between the immune response and stabilization of the mucosal barrier (23). Hence, the immune stimulation induced by LGG is an interesting finding that may prove useful in regard to oral rotavirus vaccination.

In conclusion, the results of the present study indicate that LGG potentiates gut immune responses to rotavirus and reduces the duration of acute rotavirus diarrhea. Furthermore, it is more effective than the other lactic acid bacteria preparations we studied. When LGG becomes a commercially available preparation, it should be recommended for the adjunct therapy of rotavirus gastroenteritis in previously healthy children. Because *L. casei*, particularly subsp. *rhamnosus*, has been implicated in systemic infections in severely ill patients (36), the use of LGG for the management of diarrhea in immunocompromised children cannot be recommended at present but needs further careful evaluation.

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